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Rocky Mountain Spotted Fever in Argentina

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Abstract. We describe the first molecular confirmation of *Rickettsia rickettsii*, the cause of Rocky Mountain spotted fever (RMSF), from a tick vector, *Amblyomma cajennense*, and from a cluster of fatal spotted fever cases in Argentina. Questing *A. cajennense* ticks were collected at or near sites of presumed or confirmed cases of spotted fever rickettsiosis in Jujuy Province and evaluated by polymerase chain reaction assays for spotted fever group rickettsiae. DNA of *R. rickettsii* was amplified from a pool of *A. cajennense* ticks and from tissues of one of four patients who died during 2003–2004 after illnesses characterized by high fever, severe headache, myalgias, and petechial rash. The diagnosis of spotted fever rickettsiosis was confirmed in the other patients by indirect immunofluorescence antibody and immunohistochemical staining techniques. These findings show the existence of RMSF in Argentina and emphasize the need for clinicians throughout the Americas to consider RMSF in patients with febrile rash illnesses.

INTRODUCTION

Argentina encompasses ~2,780,400 km^2 (1,073,500 mi^2) and is the second largest country in South America in population and area (www.latinamericabureau.org). Cases of epidemic typhus have been reported from Argentina since 1919[1]; however, no confirmed cases of spotted fever rickettsiosis in this country were documented until 1999.[2] We describe the first molecular detection of *Rickettsia rickettsii*, the etiologic agent of Rocky Mountain spotted fever (RMSF), from a tick vector, *Amblyomma cajennense* (the Cayenne tick; Figures 1A and 1B), and a patient with fatal RMSF from Argentina.

MATERIALS AND METHODS

Tick collection and evaluation. During October 1999, questing ticks were collected from vegetation using flannel cloth flags from several rural locations in the Departments of El Carmen, Ledesma, and Santa Bárbara in Jujuy Province in northwestern Argentina, at or near sites of presumed or confirmed cases of spotted fever rickettsiosis that had been reported to health authorities during 1993–1998. Ticks were stored in 70% ethanol at room temperature and identified by phenotypic characteristics and standard taxonomic keys. For molecular analyses, individual adult specimens, or pools of 5–15 nymphal stage ticks, were removed from the ethanol solution and allowed to air dry. The ticks were frozen in liquid nitrogen and crushed with a sterile Teflon pestle. Total DNA was extracted from pulverized ticks using an IsoQuick Nucleic Acid Extraction Kit (ORCA Research, Bothell, WA) and eluted in a final volume of 50 μL.

Two microliters of each DNA extract was screened using a polymerase chain reaction (PCR) assay designed to amplify a segment of the rickettsial 17-kd antigen gene, using 1 μmol each of primers 1 and 2, as described.[3] Positive samples were evaluated with additional molecular tests that included PCR amplification of a segment of the rickettsial *ompA* gene, using 1 μmol each of primers Rr190k.71p and Rr190k.720n,[4] and the r*poB* gene, using 1 μmol each of primers RC1600D and RC2030R.[5] PCR products were purified with a QIAquick PCR Purification Kit (QIAGEN, Valencia, CA). Duplicate sequencing reactions were performed with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). PCR primers and excess dye were removed with a DyeEx 2.0 column (QIAGEN). Sequences were determined using an ABI 3100 capillary sequencer (Applied Biosystems). Sequences were assembled with Seqmerge (Accelrys, San Diego, CA) and compared with those in GenBank using the BLAST 2.0 program (NCBI, Bethesda, MD).

Patient evaluation. During 2003–2004, four patients presented to hospitals in Jujuy Province with rapidly fatal, rash-associated, febrile illnesses. Routine blood cultures and serologic assays for dengue, leptospirosis, and hantavirus infection failed to identify a causative agent. Subsequent evaluation of clinical, epidemiologic, and autopsy data suggested that these patients died from a spotted fever rickettsiosis. Serum samples obtained during the patients' illnesses and tissue specimens obtained at autopsy were sent to the Centers for Disease Control and Prevention (CDC) for confirmatory laboratory tests.

Serum specimens were evaluated for IgG antibodies reactive with *R. rickettsii* by using an indirect immunofluorescence antibody (IFA) assay.[2] Antibody titers were interpreted as the reciprocal of the last dilution of the serum sample showing reactivity with a fluorescein isothiocyanate–conjugated goat anti-human IgG (γ-specific) at a dilution of 1/150.

Tissue specimens obtained at autopsy were fixed in 10% neutral-buffered formalin. Some tissues from one patient were also placed in 70% ethanol. Three-micrometer sections cut from formalin-fixed, paraffin-embedded, tissue samples were stained with hematoxylin-eosin and using an immunoalkaline phosphatase technique with a polyclonal rabbit anti-*R. rickettsii* antibody at a dilution of 1/500.[2] DNA was extracted from small pieces of ethanol-fixed tissue using a QIAgen Mini Kit (QIAGEN) and eluted in a final volume of 200 μL. DNA extracts were evaluated using PCR assays designed to amplify a segment of the rickettsial 17-kd antigen gene or the *ompA* gene. For each 50-μL reaction, 5 μL of extract was added to 45 μL of PCR core reagents using the High Fidelity PCR Master kit (Roche Diagnostics, Indianapolis, IN). Primers Tz-15 and Tz-16[6] were used for amplification of the 17-kd antigen gene, and primers 190-70[7]

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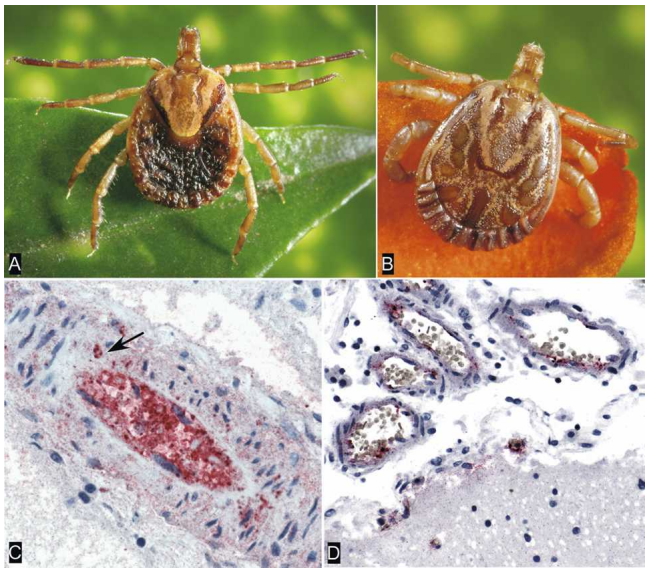

FIGURE 1. Adult female (**A**) and male (**B**) *A. cajennense* (the Cayenne tick), a vector of RMSF in Latin America. Abundant spotted fever group rickettsiae and rickettsial antigens (red) are stained by IHC in tissues of a patient with fatal RMSF, in vascular smooth muscle cells of a hepatic arteriole [arrow] (**C**), and in endothelial cells of small vessels in the cerebellar leptomeninges (**D**). The involved vessels show no appreciable inflammatory cell infiltrates despite extensive infection with large numbers of rickettsiae. Immunoalkaline phosphatase stain with fast red-naphthol phosphate and hematoxylin counterstain (polyclonal anti-*Rickettsia rickettsii* antibody at 1/500); original magnifications, ×100 (**C**) and ×50 (**D**).

and 190-701[8] for the *ompA* gene, each at a final concentration of 300 nmol. Thermal cycler parameters consisted of an initial denaturation period of 2 minutes at 94°C, followed by 40 cycles of 15 seconds at 94°C, and then 30 seconds at 55°C for the 17-kd antigen gene or 60°C for the *ompA* gene, 45 seconds at 72°C, and a 5-minute extension period at 72°C.

RESULTS

A total of 16 adult and 100 nymphal stage *Amblyomma* spp. ticks, including 9 adult and 60 *A. cajennense* nymphs, were collected from 12 sites in Jujuy Province. One adult male Cayenne tick was deposited in the US National Tick Collection at Statesboro, GA (voucher specimen accession number RML 123729). From the remaining *A. cajennense* specimens, one pool of five nymphs produced a 208-bp amplicon using the 17-kd antigen gene PCR assay that showed 100% homology to the corresponding segment of the 17-kd gene of several *Rickettsia* spp. in GenBank, including *R. rickettsii* (accession no. AY281069). A 608-bp segment of the *ompA* gene was subsequently amplified from this same extract using the *ompA* PCR assay and showed 100% identity to only the corresponding sequences of *R. rickettsii* (U43804, M31227). These ticks were collected at Saladillo, in the Department of Santa Bárbara, near the home of two children who had died of spotted fever in 1994.[2] From this same pool of specimens, a 459-bp segment of the *rpoB* gene was amplified (DQ870673) using the *rpoB* PCR assay and showed 100% homology to only the corresponding sequence of *R. bellii* (CP000087).

During September and October 2003–2004, three boys and one man from the Departments of San Salvador de Jujuy and Santa Bárbara in southern Jujuy Province, died after illnesses of ~1 to 1.5-week durations (Table 1). Patients 1, 2, and 3 became ill within a 2-week interval and resided within 5 km of each other in the town of Tilquiza, ~30 km north of the provincial capital, San Salvador de Jujuy. Patient 4 resided in the town of Palma Sola, ~20 km north of Saladillo. The patients presented for initial medical attention a median of 4.5 days after the onset of symptoms. All described one or more tick bites shortly before illness onset and all developed fever, severe headache, malaise, myalgias, and a petechial rash that started on the extremities and spread centrally to involve the trunk. Three patients became severely hypotensive and one patient developed renal failure and pleural and pericardial effusions. Two patients had elevated serum aspartate aminotransferase levels. All patients developed mild to severe hyponatremia (lowest serum sodium level, 122–133 mmol/L) and severe thrombocytopenia (lowest platelet count, 5–20 × 10^9/L). A hemorrhagic diathesis (retinal hemorrhage or intracranial bleed) was described for three patients. All patients had convulsions and became comatose shortly before death.

Serum specimens were available for Patients 2 and 3, who had IgG antibody titers reactive with *R. rickettsii* of 128 and 256, respectively. A histopathologic evaluation of tissues from Patients 1 and 4 showed lymphohistiocytic portal triaditis and Kupffer cell erythrophagocytosis in the liver, interstitial pneumonitis with pulmonary edema and hemorrhage, and focal myocarditis. No conspicuous inflammatory cell infiltrates were evident in central nervous system tissues of either patient. The immunohistochemical (IHC) stain showed abundant spotted fever rickettsiae in the endothelium of arterioles, venules, and small arteries and veins of all tissues examined, including cerebral cortex, cerebellum, liver, lung, kidney, spleen, and skin. Rickettsiae were also identified in the smooth muscle of small vessels of several tissues including lung, kidney, and liver (Figure 1C). Many of the involved vessels, particularly leptomeningeal venules and arterioles, showed no appreciable inflammatory cell infiltrates despite extensive infection with large numbers of rickettsiae (Figure 1D).

A 208-bp amplicon, amplified from ethanol-preserved frag-

TABLE 1

Demographic, epidemiologic, and clinical features of confirmed and probable cases of fatal Rocky Mountain spotted fever in Jujuy Province, Argentina, 2003–2004

Patient	Age (years)/sex	Town/department	Month/year of onset	Days onset to medical care	Days onset to death	Antibiotic therapy	Confirmatory tests
1	8/Male	Tilquiza/San Salvador de Jujuy	09/03	2	7	Cefotaxime	IHC
2	31/Male	Tilquiza/San Salvador de Jujuy	10/03	5	9	Ceftriaxone/clarithromycin	IFA
3	16/Male	Tilquiza/San Salvador de Jujuy	10/03	7	8	Ceftriaxone/ciprofloxacin	IFA
4	3/Male	Palma Sola/Santa Bárbara	10/04	4	7	Amoxicillin/chloramphenicol	IHC, PCR

ments of lung, spleen, liver, heart, and cerebral cortex of Patient 4 using the 17-kd antigen gene PCR assay, matched the corresponding sequences of several *Rickettsia* spp., including *R. rickettsii*. A 590-bp fragment of the *rompA* gene was subsequently amplified from the spleen of this patient that showed 100% homology to only the corresponding segment of the *rompA* gene of *R. rickettsii*.

DISCUSSION

These findings show conclusively the occurrence of RMSF in Argentina. Confirmed human infections with *R. rickettsii* have been documented in Brazil and Colombia for > 70 years[9–11]; however, no cases of spotted fever were described from other South American countries until the end of the 20th century.[2] Epidemic typhus has been reported from several regions of Argentina, including Jujuy Province, since the 1920s and 1930s.[1] It is likely that some early reports of "typhus" in Argentina in fact represented cases of spotted fever, similar to those cases of RMSF misidentified as epidemic or murine typhus in the United States and other regions of South America during the early 20th century.[11–13] A cluster of severe spotted fever in six young children occurred in southeastern Jujuy Province and neighboring Salta Province during November 1993 to March 1994 and resulted in the death of two patients. Presumably, these patients were also infected with *R. rickettsii*; however, only spotted fever group–specific assays were used to diagnose cases from that outbreak.[2]

Spotted fever rickettsiae other than *R. rickettsii* have been identified previously in *Rhipicephalus sanguineus*, *Amblyomma neumanni,* and *Amblyomma parvum* ticks collected in Argentina,[14–16] and *R. rickettsii* has been isolated in culture or detected by molecular methods from naturally infected *A. cajennense* collected in Brazil, Mexico, and Panama[17–19]; however, this is the first report to identify *R. rickettsii* in a tick from Argentina. The Cayenne tick is extensively distributed throughout northwestern Argentina, including Jujuy Province, and nymphs and adults are frequently identified from tick bite surveys of humans.[2,20–22] In Argentina, nymphs are most abundant from August through November and adults from November though January.[21] These intervals correspond to the months during which cases of spotted fever have been reported in Jujuy Province.[2]

Investigators first recognized the vector potential of *A. cajennense* by showing transovarial and trans-stadial transmission of *R. rickettsii* in Cayenne ticks during the early 1930s.[23–25] Epidemiologic surveys subsequently identified *R. rickettsii*–infected ticks at localities of presumed exposures and attached to patients with spotted fever.[26,27] We amplified DNA of *R. rickettsii* from a pool of *A. cajennense* ticks collected ~100 m from a household of two children who died of spotted fever 5 years earlier. The adult patient in this series, who died in 2004, lived within 20 km of this same site. These observations reflect one of the peculiar aspects of RMSF, namely persistent foci of infected ticks that create clustered cases of disease in families or communities.[28] In this series and in many others from the United States and Latin America, multiple cases of RMSF occurred simultaneously in the same household, causing as many as six deaths of affected family members over the course of several days to weeks.[2,11,29–32] Children make up a disproportionate number of the patients and deaths in these clusters. This unfortunate characteristic likely represents common exposures to tick-infested habitats during outdoor play.

The high case-fatality rates (40–95%) that characterize historic and contemporary outbreaks of RMSF in Argentina,[2] Brazil,[29–31] and Colombia[11,32] rival or exceed the cumulative mortality rate described in western Montana (65%) during 1890–1920.[33] Sentinel outbreaks of RMSF, including those rediscovered after several decades of inattention, are often represented predominantly by patients with the most severe manifestations of the illness. The patients described in this report, as well as those from western Montana during the early 20th century, lived in predominantly rural areas with limited access to health care, and most presented for medical attention relatively late in the course of their illnesses. Nonfamiliarity of many physicians with RMSF, reflected by a failure to administer the recommended antibiotic therapy for this infection (i.e., doxycycline), often contributes to an adverse outcome even in those patients who seek medical attention relatively early during the disease.

The clinical tenets that ensure successful patient outcomes in RMSF, namely consideration of the diagnosis and early initiation of specific antibiotic therapy, apply to health care providers throughout the Americas. In many respects, the challenges faced by health care professionals in Latin America are even greater, where many other endemic infectious diseases clinically mimic RMSF, including dengue, yellow fever, malaria, leptospirosis, hantavirus pulmonary syndrome, and hemorrhagic fevers caused by New World arenaviruses.[34–36] Vigilance is required of public health authorities throughout the range of RMSF to articulate the early use of doxycycline in patients with a febrile rash illness, particularly in those who also describe a recent tick bite. As shown in this case series and in many others during the last century, untreated RMSF can be a devastating multi-organ system disease and has the capacity to rapidly kill its victims. Because the interval from when a patient first seeks medical attention to the point at which antibiotic therapy is most effective at minimizing morbidity and mortality can be as brief as a few days, early empiric therapy with doxycycline is an important consideration for any patient with supporting clinical features and a history of tick exposure.[32]

In this series, all patients suffered seizures and coma; however, no prominent inflammatory cell infiltrates were identified by histology in the central nervous system (CNS) of either of the two young patients who died after 7 days of illness and were evaluated at autopsy despite extensive rickettsial infection of vascular endothelium in these tissues. The paucity of inflammation in the CNS of these patients is in agreement with the observation by Lillie[37] that conspicuous pathologic lesions are absent or scant in the CNS of patients who die before Day 11 of illness. Recently, investigators have noted that CD8+ T cells, considered crucial for the successful immune clearance of spotted fever rickettsiae, are few or undetectable around the *R. rickettsii*–infected microvasculature in the brains of patients who die < 9 days after illness onset.[38] Indeed, most patients who succumb to RMSF die before Day 10,[39] and many will develop severe neurologic manifestations, including stupor, delirium, convulsions, cerebral edema, and coma.[40] Patients with glucose-6-phosphate dehydrogenase deficiency who are infected with *R. rickettsii* often die of fulminating RMSF (i.e., death within 3–7 days[40]), with extensive

vascular damage in the CNS that occurs independent of a localized inflammatory cell response.[41] In this study, we frequently identified infection of arteriolar smooth muscle by *R. rickettsii* in several major organs, including the liver and kidney. This distribution was first noted by Wolbach[42] almost 100 years ago; however, its role in the pathogenesis of RMSF remains to be explored. Collectively, these findings support experimental observations that direct, rickettsia-mediated endothelial injury and increased microvascular permeability can occur in the absence of a localized inflammatory cell response[43] and may be particularly evident in those patients with severe and relatively brief clinical courses.

Rickettsia rickettsii is the only spotted fever group rickettsia in the Western Hemisphere to be isolated or detected by molecular techniques from patients with a fatal spotted fever rickettsiosis.[32,36,44–47] In this context, it is likely that *R. rickettsii* caused each of the spotted fever deaths in Jujuy Province identified in this report and previously.[2] Nonetheless, the confirmatory assays (i.e., IFA and IHC) that were used to identify most of these patients do not provide a species-specific diagnosis. The antibody used in the IHC assay reacts strongly with several recognized species of pathogenic spotted fever group rickettsiae indigenous to the Western Hemisphere, including *R. rickettsii*, *R. akari*, and *R. parkeri*.[39] Other tick-borne rickettsiae, including *R. amblyommii*, *R. massiliae*, and *R. bellii*, and an incompletely characterized rickettsia designated *Rickettsia* spp. strain Argentina, have been identified recently in tick species that bite humans in Argentina.[14–16] *R. bellii* has been identified in at least seven other *Amblyomma* spp. in South America[15,48]; to our knowledge, this is the first report to document its occurrence in *A. cajennense*. We amplified DNA of this rickettsia from the same pool of five nymphs that also contained DNA of *R. rickettsii*; however, it is unknown if this represented co-infection of the same tick or separate infections in two or more ticks from that pool.

At least 15 species of ixodid ticks, including many that harbor rickettsiae of undetermined pathogenicity, are reported to bite humans in Argentina.[22] Adult and immature ticks of the *Amblyomma maculatum* tick group[49] comprised the remainder of the ticks collected during the 1999 field survey in Jujuy Province. These ticks were not evaluated by molecular assays for rickettsiae; however, all tick species in this group will bite humans, and at least two species in this complex (*A. maculatum* and *A. triste*) are putative vectors of *Rickettsia parkeri*, recently recognized as a cause of eschar-associated spotted fever rickettsiosis in the Western Hemisphere.[50–53] Eschar-associated rickettsioses after tick bites have been reported recently from Uruguay, Brazil, and Argentina.[54–56] A serosurvey of 105 persons residing in the Department of Santa Bárbara in Jujuy Province, conducted between two recognized episodes of severe spotted fever in 1994 and 2004, showed antibodies reactive with spotted fever group rickettsiae at titers $\geq$ 64 in 4% of the participants; however, none of the persons with significant antibody titers recalled an illness of the severity characteristically associated with classic RMSF.[2] These data suggest that multiple spotted fever rickettsioses of varying severity may exist in this country similar to many other regions of the world.[57]

The true, rather than collective, clinical spectrum, epidemiology, and distribution of the various spotted fever rickettsioses indigenous to the Americas will depend on the continued efforts of clinicians and laboratorians to achieve accurate diagnoses by using tissue-based molecular or culture techniques. Renewed interest in RMSF by Latin American investigators also signals a vital and exciting period of scientific exploration in this region of the world and provides hope that future discoveries will enhance clinical awareness and early presumptive diagnosis and treatment of this recalcitrant and life-threatening infectious disease.

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